GM Project Approval Process – How it Works

1. ***Decide if your work concerns:*** **GM animals, GM human viruses or viral vectors, GM microorganisms other than viruses (but including cell cultures), GM plant viruses, GM plants**

2. ***Download*** **the appropriate Risk Assessment form and the 'Signature page for GM projects' form: http://www.cardiff.ac.uk/osheu/safety/biological/index.html**

3. ***Fill in the risk assessment form*** **electronically and e-mail it to your School/Department Biological Safety Officer (BSO). They will check it/suggest any changes required. After feedback and the inclusion of any amendments - move on to points 4 and 5.**

5. ***Send an electronic copy to:***
Dr Rachel Coombe (CoombeRA@cf.ac.uk) or Mrs Heather Lam (LamH@cf.ac.uk)

The BSO will sign it and send it on to the HOS/HOD

HOS/HOD will sign it off and send it on to OSHEU

For Class 1 Projects:
Forms will be circulated to GMBA committee members

The reviewing/approval process will take approximately 1 month and the premises will be inspected by OSHEU. Note: *Projects originally submitted as Class 1 may be upgraded to Class 2 by the committee and will therefore have to follow the GM class 2 route of review.*

If the committee is satisfied that the risk assessment is a Class 1 activity, approval will be given for work to go ahead. Risk assessments will be signed – off by the chairman of the committee.

For Class 2/3 Projects

Risk assessment will be reviewed at next meeting

6. Containment level 2 projects and above will require HSE notification (additional notification form and cost)

7. For all projects download GM worker registration forms **from http://www.cardiff.ac.uk/osheu/safety/biological/index.html print out, fill in, sign and send to: Dr. Rachel Coombe at OSHEU *via* the internal mail**

[For completion by word processor. To view the guidance provided in the Comments boxes select View/Comment or View/Markup]

Genetic Modification and Biological Agents Safety Committee [GMBA]

Proposal and Risk Assessment for Work with Genetically Modified Microorganisms (including cell cultures) Other than Viruses

You should take note of the information provided in the **Comments** boxes and consult the HSE document "**ACGM Compendium of Guidance**" http://www.hse.gov.uk/hthdir/noframes/acgmcomp/acgmcomp.htm. Hard copies may also be obtained from OSHEU on request.

Date of Application: 09/05/16	**Project Number:**	
Title of Project: iGEM Project: Development of dual CRISPR-Cas9 as a diagnostic tool for the presence of pathogen-derived nucleic acids.		
Person responsible/Supervisor: Dr Geraint Parry	☎: 07411967414	**Email:** geraint@garnetcommunity.org
Department: Biosciences	**Location of work** (Building & Room No.) Sir Martin Evans Building, West 4th Floor	
Proposed final activity Class	**1 X 2** ☐ **3** ☐	

Connected Programme Information

Is this current project 'connected' to an ***existing*** project e.g. one that has been previously submitted to the Health and Safety Executive (HSE) forming a larger *Connected Programme of Work*?

Yes ☐ No X

If 'Yes', provide the following information regarding the ***existing*** project: (see Section 2 also)

HSE project number:

Cardiff University project number: GM130/

Title of project (in full):

For Office Use:		
Containment Level Required by GMSC	**1 ☐ 2 ☐ 3 ☐**	**Signature of Chairman:** **Date:**
Date Notified to HSE (if required)**:**	**Date of HSE reply:**	**HSE Reference number :**
Laboratory/Facility approved for the required Containment Level	**Date:**	**Signature of inspecting Biological Safety Adviser:**

Section 1 Personnel

Person responsible/Supervisor details **Briefly indicate your experience of working with microorganisms and genetically modified organisms and any training you have received**
Dr Geraint Parry (Primary PI). Long experience working with genetically modified organisms over the course of PhD, PDRA and previous lectureship. Has taken GM-worker courses at University of Nottingham, University of Liverpool and overseas at Indiana University in the USA. Experience working with bacteria (*E.coli, A.tumefaciens*) and plants (*Arabidopsis thaliana, Nicotiana benthamiana*).

Table 1

Names of other workers on the project (if known)	**Qualifications**	**Experience/Training**
Asal Golshaie Laura Bird David McMaster Robert Newman Andrew Brimer Christian Donohoe Nikolas Demetriou	Second Year Genetics Second Year Biology Second Year Biomedicine Second Year Genetics Second Year Biomedicine Second Year Chemistry Second Year Chemistry	1st and 2nd year University lab classes

James Long	BSc Immunology, Aberdeen 2015	University lab classes, iGEM training (Aberdeen 2014)
Amit Jathoul (Secondary PI, principle lab supervisor),	PhD	Years of lab experience at Cardiff University and UCL. Taken Cardiff GM course.
Joanne Kilby (lab manager)	PhD candidate	Years of lab experience at Cardiff University. Taken Cardiff GM course. Lead technician for Murray lab.

Section 2: Project

If the '*Yes*' box was checked in the '*Connected Programme Information*' section, please explain how this current project is connected to the existing project previously approved by the HSE **HSE Definition**: *'a connected programme of work" means a series of activities involving contained use which form a coherent and integrated programme'* (connected programme guidance document)
Explanation of connection:

Description of the current project, including the methods to be used and the purpose of the genetic modification:

1) The iGEM project will aim to develop use CRISPR-Cas as a diagnostic tool to identify pathogen-derived nucleic acids. We will use a mutant Cas9 that lacks nuclease activity, tethered to either a split luciferase or LacZ proteins. We will use these molecular tools to assess whether we can detect a foreign nucleic acid sequence in an *in-vitro* system comprised of components purified from bacterial samples. 2) Only *E.coli* containing a range of Cas9 constructs and associated clones (such as guide RNAs). 3) No hazards over those for use of lab disabled *E.coli K12*
Will you cultivate on a large scale (e.g. 10 or more litres per culture)?
Yes ☐ **No X**

Table 2

Host(s)	Vector(s)	Source of nucleic acid insert (e.g. species)	Description of DNA to be inserted (including brief explanation of function)
E.coli K12 *DH5alpha, TOP10,* ER1793	Bacterial Expression Vector such as Gateway compatible pDEST14, pDEST15	Synthesised (IDT gblock) bacterial DNA from *E.coli*	- E.coli codon optimised Cas9m-split LUC (N or C terminal portions). Planned experiment relies on split-Cas9m portions coming into close proximity, enabling LUC activity. - SpCas9m-splitLacZ (N or C terminal portions). Planned experiment relies on split-Cas9m portions coming into close proximity, enabling LacZ activity. - Range of Cas9 guide RNAs targeted against bacterial ribosome genes. - Lux Operon with modified LuxA or LuxB genes to enable colour-switch

Section 3 Risk Assessment

NB: This section should include in each of the answers to the questions sufficient detail to justify your assessments of Level of Risk. Insufficient detail is likely to result in delays in the overall application process.

Level of risk can be estimated using the matrix given in Appendix 1

Section 3A: Risk to Human Health

3A.1 Characteristics of the host and any hazards associated with it
No hazards aside from those associated with lab disabled *E.coli* K12. We will be using strains such as DH5alpha, TOP10 or ER1793 (https://goo.gl/qhmuKD) .
Level of Risk: Effectively Zero

3A.2 Characteristics of the vector system and any hazards associated with it
We will be using vectors for high expression of mutant Cas9 in *E.coli* and subsequent protein purification. Although this plasmid will allow expression in other bacteria the expression of nuclease-disabled Cas9 poses no hazard.
Level of Risk: Effectively Zero

3A.3 Source and characteristics of the inserted gene product and any hazards arising directly from its use (include an estimation of the level of expression and biological activity of the recombinant gene product)
High level of mCas9 expression. No hazard associated with the gene products as the mutated Cas9 will not modify the bacterial genome as its nuclear activity is disabled. We will use guide RNAs to direct mCas9 to a rRNA gene.
Level of Risk: Effectively Zero

3A.4 Hazards arising from the alteration of any existing traits, if applicable
No alteration of any bacteria traits. As outlined above the mCas9 will be targeted to a rRNA gene but has no nuclease activity.

Level of Risk:	Effectively Zero

3A.5 Potential hazard of sequences within the GMM being transferred to related micro-organisms
None, genetic elements are all derived from *E.coli.* In theory the Cas9 enzyme will have activity in other organisms but as the nuclease is disabled it will not modify the genome according to our current knowledge.
Level of Risk: Effectively Zero

3A.6 The overall likelihood that, in the event of exposure, the GMM could cause harm to human health (e.g. provide a worse-case scenario outcome)
The gene product would have no effect on human health as the Cas9 requires a gRNA for activity. If exposure occurs to gRNA and mCas9 there would be able no effect given the nuclease-disabled Cas9.
Level of Risk: Effectively Zero

3A.7 Assign the provisional containment level which is adequate to protect against hazards to human health

Tick required box:

1	**X**
2	☐
3	☐

Section 3B: Assessment for Environmental Harm

3B.1 What is the capacity of the GMM to survive, establish, disseminate with and/or displace other organisms?
Lab disabled E.coli including DH5alpha or TOP10. Usual mechanisms will be in place to destroy biological samples before they leave the lab.
Level of risk: Effectively Zero

3B.2 What is its ability to cause harm to animals?
The danger associated with use of lab-disabled non-pathogenic *E.coli* is effectively zero. As highlighted above there is no danger associated with the expression of a nuclease-disabled Cas9 protein.
Level of risk: Effectively Zero

3B.3 What is its ability to cause harm to plants?
E.coli has no ability to transfer genetic information to plants and does not cause an external danger.
Level of risk: Effectively Zero

3B.4 What is its ability to cause harm to other organisms?
Lab-disabled *E.coli* do not pose a threat. As highlighted above there is no danger associated with the expression of a nuclease-disabled Cas9 protein.
Level of risk: Effectively Zero

3B.5 What is the potential for transfer of genetic material between the GMM and other organisms?
Although the plasmid can transfer to environmental *E. coli* precautions will be taken to ensure that this does not occur through good microbiological practice and disinfection/sterilisation of all waste generated.
Level of risk: Effectively Zero

3B.6 Is there any hazard as a result of phenotypic or genetic instability?
Lab-disabled *E.coli* do not cause a threat. Any instability will have no effect on pathogenicity. Our planned alterations do not change the risk of phenotypic or genetic instability.
Level of risk: Effectively Zero

3B.7 Hazards arising from the alteration of any existing traits, if applicable
Alteration of trait disables the nuclease activity of Cas9. This does not create any hazard.
Level of Risk: Effectively Zero

Section 4 Control Measures Monitoring, and Inactivation

Provide details of the control measures to be used to protect human health and the environment and the means by which their use and effectiveness will be monitored. .

Are any of the procedures likely to generate aerosols? Yes ☐ No X

Will a safety cabinet be used? Yes ☐ No X

Will any procedures use sharps? Yes ☐ No X

If Yes, state control measures to be used and means of disposal:

Will liquid waste be autoclaved ☐ or disinfected X?

If Disinfected:

Product Name: Standard lab Bleach

Generic Chemical Name: 50% Sodium Hypochlorite. Aim for a final concentration of 0.5%.

Expected degree of kill: Complete

How is this validated? (e.g. is in-house testing carried out, or is there specific data available regarding efficacy against the organisms you are working with). **Explanation**: The bacterial cultures must be submerged in the hypochlorite solution for at least 20 minutes. This has been shown to effectively kill the bacteria.
http://www.uwo.ca/animal-research/doc/bleach-sop.pdf

Solid waste to be autoclaved X disposed of as clinical waste ☐

Details of any other control measures to be used:

Standard lab practice to prevent additional contamination.

Section 5: Emergency Planning

Does your project require an emergency action plan?

Yes ☐ No X

If Yes, does your School emergency action plan provide adequate protection in case of accidental release?

Yes ☐ No X

If No, give details of specific extra safety measures which will be applied to the project

None aside from closer initial supervision of inexperienced researchers.

To print this current application/risk assessment form without the *Comment* boxes by *go to File/Print/Print what/* and replacing the*, "document showing markup"* with the *"document"* selection).

Please ensure you have printed off the 'School / Department GM signature page form' from the OSHEU website and obtained the necessary signatures.

Attach the signed 'School / Department GM Project signature page form' to the project proposal/risk assessment form and send them by internal mail to: Heather Lam – OSHEU, 47 Park Place. You will also need to send an electronic copy of this current form (without attached signature form) to LamH@cf.ac.uk. *Both* electronic and hard copies are required for efficient processing of your proposal.

THE PROJECT PROPOSAL/RISK ASSESSMENT WILL NOT BE REVIEWED BY THE GMBA UNTIL OSHEU HAS RECEIVED BOTH THE ELECTRONIC AND HARD COPY VERSIONS OF THE DOCUMENT.

Review of Risk Assessment

NB: This risk assessment must be subjected to regular review by the supervisor and also be reviewed if project circumstances change significantly. The GMBA will request a biannual review as part of this requirement.

Appendix 1: Matrix for estimation of level of risk

[Consequence x likelihood = risk of causing harm]

	Likelihood of Hazard			
Consequence of Hazard	**High**	**Medium**	**Low**	**Negligible**
Severe	High	High	Medium	Effectively Zero
Medium	High	Medium	Medium/Low	Effectively Zero
Low	Medium/Low	Low	Low	Effectively Zero
Negligible	Effectively Zero	Effectively Zero	Effectively Zero	Effectively Zero